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⑤4血液稀釈用試料ビーカの製法

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明　細　書

1. 発明の名称

血液稀釈用試料ビーカの製法

2. 特許請求の範囲

内壁を清浄されたポリエチレン、ポリプロピレンもしくはガラス等の材質の容器を、ポリビニルアルコール、ポリビニルピロリドン、あるいは液状ナイロンの水溶液中に所定の時間浸漬せしめ、しかる後に室温以上180°Cまでの温度内で急激に乾燥させ、内壁に血球保護物質膜を形成せしめることを特徴とする血液稀釈用試料ビーカの製法。

3. 発明の詳細な説明

本発明は自動血球計数器に用いる血液稀釈用試料ビーカの製法に関する。

本発明に類するビーカに関してはすでに実公昭49—42225で述べられているが、その後実験を重ねた結果、不都合な面が生じて来たので改良を加え、良好な結果が得られたのでその詳細について説明する。

従来、自動血球計数器で血球を計数するには、血液を生理食塩水等の稀釈液で高倍率に稀釈し、一個ずつ計数可能な程度の稀釈濃度にして用いているが、血球が長時間もとの形状を維持させるために稀釈液のPH，滲透圧等を血液と同等のものとする必要があり、稀釈液としては生理食塩水と同等の組成を有し、かつ血球程度の大きさのゴミを除去したものが用いられている。しかし、稀釈液の組成を血液のものと等しくしても、高倍率に稀釈すると、血球が溶血を起し、血球が時間とともに減少してしまう。例えば、従来から良く用いられているガラスビーカに5万倍に稀釈した血液を入れておくと、稀釈液の温度や血液によつて多少の差はあるが30分後には数パーセントの減少が認められ、計数結果に重大な影響を与える。

以上の影響を軽減するために高密度ポリエチレン樹脂のコップ状成形体内壁にアルブミンの被膜を附着させたビーカを用いると、血球数の減少が大幅に食い止められたが、その後実験を進めるうちに、アルブミン被膜は、長期間の保存に対しては、変性が起り効果が薄れてしまうことがわかつ

た。又、多検体の血液で実験するうちに、血液によつては壁面のアルブミンの溶出により、赤血球の分化現象を誘発し、計数結果に異状をきたす現象がまれに起ることがわかつた。その他の異状現象として血球が壁面に吸着したり、粘着したりすることも起るなど、少数の血液に対してはアルブミンを用いることは不適当であるという欠点があつた。

本発明は前記欠点を解消するものであり、いかなる血液に対しても、血球の保護効果が薄れず、長時間安定に血球を保護するような血液稀釈用試料ビーカの製造方法を提供する。

以下、実験結果に基づいて本発明実施例を説明する。血球の保護物質としては、アルブミンなどの変性しやすい物質以外には、ポリビニルアルコール、ポリビニルピロリドン、ナイロンなどのように高分子重合体物質で、微少量だけ水に溶け出す物質がある。ポリビニルアルコールやポリビニルピロリドンは常温では水に溶けにくいので、粉末を温湯に徐々に溶解させることができる。ナイロンについては、液状ナイロンを用いる。以上の高分子重合体物質の水溶液に内壁を清浄されたポリエチレン、ポリプロピレンもしくはガラス等の材質で成形されたビーカを浸漬させ、所定の時間後に室温以上180°C までの温度内で急激に乾燥させる。

以上のようにしてビーカの内壁に保護物質膜を形成させたビーカと、無処理のビーカについて比較したものが第1図に示されているグラフであり横軸は稀釈後の放置時間を取り、縦軸には血球の減少率を取つている。実験では1立方ミリメートル当り、500万個前後の赤血球を有する血液を500万倍に稀釈したものであり、グラフ1は、PVA（ポリビニルアルコール）0.01％の水溶液に、グラフ2は同じく0.1％の水溶液に、さらにグラフ3は同じく0.5％の水溶液に五分間以上浸漬させ乾燥させたビーカを用い、グラフ4はアルブミン0.1％の水溶液に五分間以上浸漬させ乾燥させたビーカを用いたものであり、グラフ5は未処理のビーカを用いている。グラフから明らかなように、未処理のビーカを用いると、4時間で計数値が半分になつてしまうが、処理したビーカでは血球数は減少しない。但し、PVA 0.01％の水溶液では、処理が不完全であり、0.1％以上の水溶液での処理が必要であるが、0.5％で処理したビーカの内壁には一部被膜が剥離状態にあるものが認められるため、0.1％の水溶液が適当である。他のPVP（ポリビニルピロリドン）や、液状ナイロンでもほぼ同様の結果が得られた。

さらにビーカを水溶液に浸漬する時間について行つた実験結果を第2図に示す。第1図と同様に横軸に稀釈後の放置時間を取り、縦軸に赤血球の減少率を取つた。グラフ5は未処理のビーカについてのものであり、グラフ6は、PVAの0.1％水溶液に瞬間浸漬させたものであり、グラフ7は1分間浸漬させたものであり、グラフ8は5分以上浸漬させたものである。この実験からも明らかなように、短時間の処理でも、未処理のビーカと比較すると、一定時間は効果があるが、一定時間を経過すると、急激に血球数が減少する。5分以上浸漬したものについては、稀釈後4時間経過しても、血球数の減少は認められない。PVAのほか、液状ナイロンについても同様な結果が得られた。

次に乾燥時の温度については、室温程度の低い温度でゆつくり乾燥させると、比較的薄い皮膜が形成され、血球の保護効果はそれ程著しく減退はしない。この方法は薄手のビーカに皮膜を形成させる場合、皮膜がはがれにくいという利点があるが、乾燥時間が長くかかり、ゴミ等の附着が生じやすく好ましくない。しかし温度を上げすぎるとポリエチレン、ポリプロピレン等のビーカが変形するおそれがあり、又PVA，PVP等が変性してしまう可能性があり、上限は180°C 程度までである。

以上のようにして皮膜を形成したビーカに、血液の稀釈溶液を注入すると、壁面の皮膜が、ごくわずかだけ溶出するが、すべて溶出することはなく、ごくわずかだけ溶出した保護物質が、血球の表面に保護膜を形成し、そのために壁面との衝突

によつて血球が破壊されることはなく、又壁面に血球が粘着したりすることはなく、血球が固定された状態で保存されるため、長時間の放置においても血球の破壊が起らず、血球数の変動が起りにくくなる。又、PVA，PVP，液状ナイロン等の長期にわたつて安定な物質を用いたため、ビーカの長期の保存に対して効果は薄れず、又、これらの物質は血球に対しては、不活性であるためにアルブミンのように血球の分化などの現象を誘発するおそれがなく、いかなる血球に対しても効果は大きい。

また上記物質はガラス，ポリエチレン，ポリプロピレン等、ビーカの材料の如何、又軟質，硬質等の材料の性質の如何にかかわらず効果を発揮するために、現在血液稀釈用ビーカとして用いられているビーカに対しては皮膜を形成させることができるため、多くの血液検体を処理する病院の検査室や、検査センターなどで本発明による試料ビーカを用いることは非常に効果的である。

4. 図面の簡単な説明

第1図および第2図は、種々のビーカについて時間に対する血球数の減少率をグラフ化したものである。

1 … PVA 0.01 %で処理、2 … PVA 0.1 %、3 … PVA 0.5 %、5 … 未処理ビーカ、6 … 瞬間浸漬処理、8 … 5分以上浸漬処理

特許出願人

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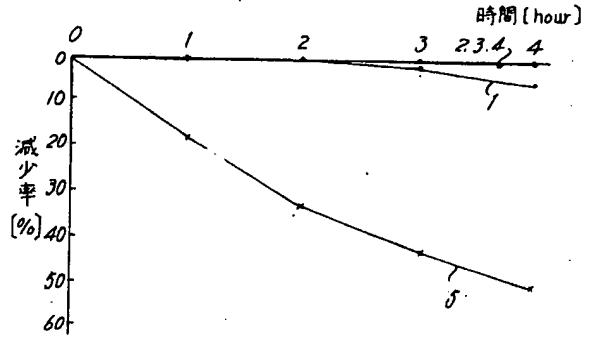
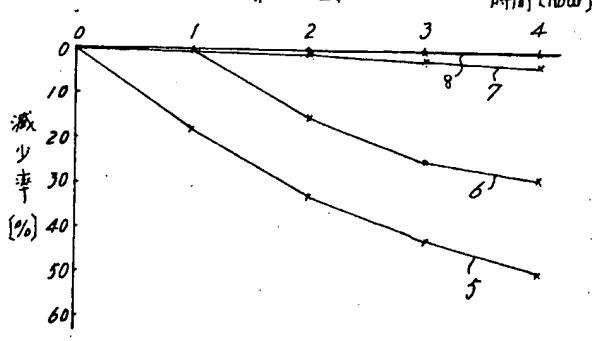
第1図



第2図



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(54) Title of Patent: Method for producing sample beakers for diluting blood

(21) Patent Application No.: 51-10622

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Specifications

1. Title of Invention:

Method for producing sample beakers for diluting blood

2. Claim:

A method for producing sample beakers for diluting blood, characterized in that a container made of a material such as polyethylene, polypropylene, glass, etc., with a cleaned inner wall is immersed for a specific time in an aqueous solution of polyvinyl alcohol, polyvinyl pyrrolidone, or liquid nylon, after which it is rapidly dried at a temperature from room temperature to 180°C, forming layer of a substance which protects blood cells on the inner wall.

3. Detailed Explanation of Invention:

This invention concerns a method for producing sample beakers for diluting blood which are used in automated blood cell counters.

Beakers of the kind of this invention have already been mentioned in Utility Model Bulletin No. 49-42225, but as a result of performing later experiments, it was found that unsuitable surfaces were produced, and improvements were made, which gave good results. The details will be explained below.

Up to now, in counting cells with automatic blood cell counters, the blood was diluted at high dilution rates with diluents such as physiological saline to concentrations such that the cells could be counted one at a time. However, it was necessary to make the pH, osmotic pressure, etc., of the diluent the same as those of blood so that the original shapes of the cells could be preserved for long periods of time. Solutions which have compositions equivalent to physiological saline and with foreign particles about the size of blood cells removed have been used as diluents. However, even though the composition of the diluent is made equivalent to blood, when it is diluted to a high dilution rate, the blood cells cause hemolysis and are reduced, over time. For example, when blood diluted 50,000 times is put into the glass beakers which have been frequently used up to now, reductions of several percent are observed after 30 minutes, although the percentages differ somewhat depending on the blood and the temperature of the diluent; this has a great effect on the results of the counting.

In order to reduce this effect, cup-shaped beakers made of high-density polyethylene resin with albumin films on their inner walls have been used. By using them, the reduction in the number of cells was largely held down, but when further experiments were performed, it was discovered that the albumin films degenerated when stored for long periods, reducing their effectiveness. Moreover, while experimenting with many blood samples, it was discovered that a phenomenon of differentiation of the red blood cells was occasionally induced by elution of the albumin on the wall surfaces, causing abnormal counting results. Another abnormal phenomenon was that of cells being adsorbed on the wall surfaces or sticking to them. Thus, using albumin had the drawback that it was unsuitable for a small number of blood samples.

This invention solves this problem. It provides a method for producing sample beakers for diluting blood of a kind such that the effectiveness of the protection of the blood cells is not reduced and they are protected in a stable manner over long periods no matter what blood samples are used.

Working examples of this invention will be explained below, on the basis of experimental results. Besides substances which degenerate easily, such as albumin, substances which protect blood cells include high-molecular-weight polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, nylon, etc.; they only dissolve in water in very small quantities. Since polyvinyl alcohol and polyvinyl pyrrolidone do not dissolve readily in water at ordinary temperatures, the powder can be dissolved gradually in hot water. For the nylon, liquid nylon can be used. A beaker made of a material such as polyethylene, polypropylene, or glass, etc., with an inner wall which has been cleaned, is immersed in an aqueous solution of such a high-molecular-weight polymer and, after a specific time, dried rapidly at a temperature from room temperature to 180°C.

Fig. 1 shows the results of comparing a beaker with a protective film formed on its inner wall as described above and an untreated beaker. The horizontal axis shows the time the diluted solution was left standing and the vertical axis the percentage of reduction of blood cells. In the experiment, blood containing approximately 5,000,000 red blood cells

per cubic millimeter was diluted 5,000,000 times. Graph 1 shows the results obtained when a beaker was immersed for more than 5 minutes in a 0.01% aqueous solution of PVA (polyvinyl alcohol) and dried; Graph 2 shows the results when a 0.1% solution was used, and Graph 3 shows the results when a 0.5% solution was used. Graph 4 shows the results when a 0.1% solution of albumin was used, and Graph 5 shows the results when an untreated beaker was used. As is clear from the graphs, when the untreated beaker was used the count was reduced by half in 4 hours, but the cell count was not reduced with the treated beakers. However, the treatment with 0.01% PVA was incomplete; treatments with 0.1% or higher solutions were needed, but since it was observed that the film was partially peeled off the inner wall of the beaker treated with an 0.5% solution, it was found that the 0.1% solution was suitable. Almost the same results were obtained with PVP (polyvinyl pyrrolidone) and liquid nylon.

Furthermore, Fig. 2 shows the experimental results for the times for which the beakers were immersed in the aqueous solution. As in Fig. 1, the horizontal axis shows the time the diluted solution was left standing and the vertical axis the percentage of reduction of blood cells. Graph 5 shows the results with an untreated beaker, Graph 6 shows the results when the beaker was immersed for a moment in a 0.1% PVA solution, Graph 7 shows the results when it was immersed for 1 minute, and Graph 8 shows the results when it was immersed for more than 5 minutes. As is clear from this experiment, the beakers treated for specific immersion times, even when momentary, were more effective than the untreated beaker, but the cell counts decreased rapidly as the time passed. No decrease in blood cells was observed when the beaker was immersed for more than 5 minutes, even after 4 hours elapsed after the dilution. Besides PVA, the same results were obtained with liquid nylon.

Next, concerning the temperature during drying, when the film was dried slowly at a low temperature, that is, around room temperature, a comparatively thin film was formed, and the effect of protecting the blood cells was not very great. This method has the advantage that the film does not peel easily when a thin film is formed on the beaker, but the drying time becomes longer and adhesion of foreign particles, etc., occurs readily; therefore, this is not desirable. However, if the temperature is raised too much, there is a possibility of polyethylene, polypropylene, etc., beakers being deformed, and the PVA, PVP, etc., may degenerate. The upper temperature limit is about 180°C.

When a diluted blood sample is put into a beaker in which a film was formed in the way described above, the film on the wall surface dissolves to a very slight extent, but it does not entirely dissolve. The slightly eluted protective substance forms a protective film on the surfaces of the blood cells; therefore, the blood cells are not destroyed by collisions with the wall surface and the blood cells do not stick to the wall surface. Since the blood cells are kept in an unchanged form, they are not destroyed even when left for a long time and variations in the cell count do not easily occur. Furthermore, since substances which are stable over long periods, such as PVA, PVP, liquid nylon, etc., are used, their effectiveness is not reduced when the beakers are kept for long periods, and since these substances are inert with respect to the blood cells, phenomena such as differentiation of blood cells cannot occur, unlike the case of albumin. Thus, the invention is effective with any kind of blood sample.

Furthermore, since the effects of the invention are exhibited regardless of the materials used for the beaker, including glass, polyethylene, polypyrrolidone, etc., and whether the materials are hard or soft, the films can be formed on beakers which are presently used for blood dilution. Therefore, using the sample beaker of this invention in examining rooms of hospitals, examining centers, etc., where large numbers of blood samples are processed, is very effective.

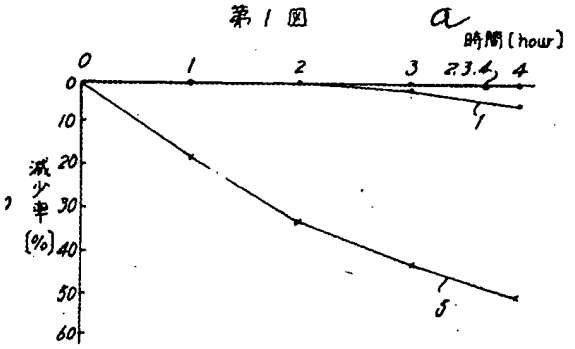
4. Simple Explanation of Drawings:

Figs. 1 and 2 show graphs of the percentages of decrease of blood cells with time for various beakers.

1 ... Treated with 0.01% PVA, 2 ... 0.1% PVA, 3 ... 0.5% PVA, 5 ... untreated beaker, 6 ... momentary immersion, 8 ... immersed for more than 5 minutes

Applicant: Toa Medical Electronics Co., Ltd.

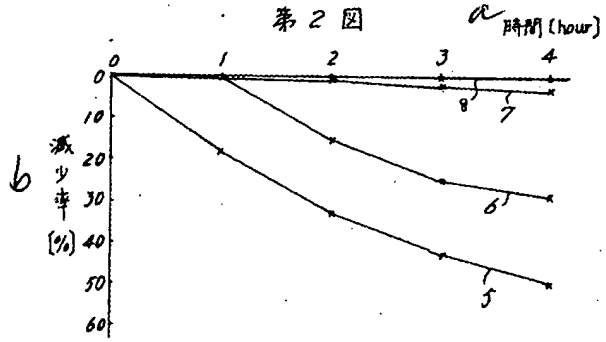
Fig. 1



a. Time (hours)

b. Reduction percentage (%)

Fig. 2



a. Time (hours)

b. Reduction percentage (%)